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hprt Mutation frequency among workers exposed to 1,3-butadiene in China

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Abstract

Hypoxanthine-guanine phosphoribosyl transferase (*hprt*) mutation frequency (M_f) was studied in workers at a polybutadiene rubber production facility in Yanshan, China. Exposed workers included for study were active either as process analysts, who sampled butadiene production process lines and analyzed product by gas chromatography, or as process operators, who did routine process control, minor maintenance and, as needed, major repair operations. For process analysts at the polymer and dimethyl formamide (DMF) facilities, the median air levels of BD were 1.0 and 3.5 ppm, respectively. Among process operators, air levels of 1.1 ppm were found during routine activities, while the median air level during pump repair and related operations was 45 ppm (6-h time-weighted average). Overall, M_f was similar in unexposed (mean $M_f = 20.2 \times 10^{-6}$) and butadiene-exposed (mean $M_f = 21.6 \times 10^{-6}$) workers ($P = 0.68$). M_f decreased with cloning efficiency, increased with age, and was moderately greater in women than in men. After adjustment by multiple regression analysis for mean age, sex, and cloning efficiency, the adjusted mean M_f (X_{adj}) was 13.6×10^{-6} in unexposed and 18.0×10^{-6} in butadiene-exposed. This 32% difference was, however, not statistically significant ($P = 0.13$). Butadiene exposure was associated with a modest, if any, increase in *hprt* M_f in this population of Chinese workers.

Keywords: *hprt* mutation; 1,3-Butadiene; Occupation

1. Introduction

The highly reactive flammable gas, 1,3-butadiene (BD), was first produced in large volumes during World War II for use in the production of synthetic rubber. Currently, more than 5 million tons of BD are produced annually worldwide, about 1.5 million tons of which are used in the United States (Morrow, 1990). Approximately 65 000 U.S. workers may be exposed annually to BD (Fajen et al., 1990). While it is commonly used in the production of rubber and thermoplas-

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tic resins, BD has also been found in automobile exhaust, cigarette smoke, and in community air at the perimeter of manufacturing plants (International Agency for Research on Cancer, 1992).

BD is genotoxic and acts as an animal carcinogen, but evidence for carcinogenicity in humans is limited (International Agency for Research on Cancer, 1992). Extrapolation from animal data on BD to risk in humans has been controversial because of uncertainties regarding uptake and metabolism in humans vs. experimental animals (Bond et al., 1995; Melnick and Kohn, 1995). We undertook to characterize exposure to BD at a butadiene polymer production facility and to relate this exposure to cytogenetic and mutagenic effects in humans. The determination of in vivo somatic cell mutations in humans exposed to suspect carcinogens provides a method to assess short-term biologic effects that may be related to human carcinogenesis. Increased frequency of mutations in the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus (Albertini et al., 1990) have been described in a U.S. occupational group exposed to BD (Ward et al., 1994). Here we describe the frequency of occurrence of mutations in *hprt* among Chinese workers exposed to BD.

2. Methods

Workers were studied at a polybutadiene rubber production facility at Yanshan, China. The purification of BD from an initial hydrocarbon stream occurred at two sites: the DMF facility, where initial distillation and extraction occurred using a proprietary dimethyl formamide (DMF) process; and the recovery facility, where final distillation occurred. Polymerization and packing of the final product took place in the polymerization facility; the remaining unpolymerized material was returned to the recovery facility for further processing.

Because the production process was enclosed, general environmental emissions were limited. On an initial visit to the facility, three groups of workers with high potential exposure were identified. DMF process analysts sampled process lines and analyzed product by gas chromatography at the DMF unit, while polymer process analysts carried out these tasks at the recovery and polymerization units. The third group of exposed workers were process operators at the recovery facility who carried out routine minor maintenance and, as needed, major repair operations. After the purposes of the study and procedures were explained and informed consent was obtained, 41 exposed workers were included for study. For comparison, 40 unexposed subjects were enrolled. The unexposed subjects were age (5-year) and gender matched in groups to the exposed. Upon review of occupational histories, two controls, who were determined to have worked with BD in the past, were excluded from analysis. The study groups are presented in Table 1.

The study took place during March 1994 and was staffed by Chinese and U.S. scientists. Subjects completed a brief questionnaire, administered by study staff, regarding work history, selected medical conditions, and tobacco use. During the 6-h work shift, personal samplers were used for collecting air at the breathing zone, by drawing atmospheres through charcoal tubes using individual pumps. The samples were analyzed by gas chromatography, using an adaptation of the NIOSH method 1024 (National Institute for Occupational Safety and Healt, 1987), and expressed as parts per million (ppm), as a 6-h (full work-shift) time-weighted average

Table 1
Study subjects (Yanshan, China, 1994)

	Unexposed	Butadiene-exposed			
		DMF	Polymer	Recovery	Total
Male	14	0	0	15	15
Female	24	10	15	1	26
Total	38	10	15	16	41

(TWA). In addition, numerous grab samples were taken at the breathing zone using 50-ml glass syringes during the study and analyzed on-site, using a portable Photovac/reg/ gas chromatograph.

A post-shift blood sample (8 ml) was collected, from which mononuclear cells were isolated over a LeucoPREP/reg/ tube with Ficoll density gradient liquid and polyester gel (Becton and Dickinson, Lincoln Park, NJ). Mononuclear cells were washed in RPMI medium (UCSF, San Francisco, CA) with 10% fetal bovine serum (FBS) (Gemini Bioproducts), frozen in RPMI with 42% FBS and 8% dimethylsulfoxide (DMSO) under a rate-controlled (1°C/min) condition, and stored as viable cells in gas-phase N_2. Blood samples (19 ml) were fractionated (serum, plasma, red blood cells, and buffy coat) and stored. Whole blood cultures were established for cytogenetic studies.

The T-cell cloning assay has been described (Albertini et al., 1990). For mutation frequency determination (M_f), thawed cells were incubated in medium containing 1 μg/ml PHA (HA17; Wellcome Diagnostics) for 36–40 h to achieve mitogen stimulation. Washed cells were then plated in growth medium (RPMI 1640 containing 20% nutrient medium HL-1, 5% defined supplemented bovine calf serum, 10–20% LAK supernatant containing 0.125 μg/ml PHA, and 1×10^4 irradiated human lymphoblastoid feeder cells/well. After 10–16 days' incubation, growing colonies were determined by use of an inverted phase contrast microscope. The cloning efficiencies (CE) are calculated by Poisson relationship, $CE = -\ln P_o/x$, where P_o is the fraction of wells negative for colony growth and x is the average number of cells originally inoculated per well. The thioguanine selected CE divided by the mean unselected CE yields the M_f.

Nonparametric procedures were used for univariate statistical analyses; the Spearman correlation test, the Mann-Whitney U-test for independent samples, and the χ^2 test. For multivariate analyses, linear regression analyses were carried out on the natural log (ln) of M_f (SPSS, 1993).

3. Results

As shown in Table 2, butadiene-exposed workers were, on average, somewhat younger than the unexposed comparison group. None of

Table 2
Selected characteristics of study subjects

	Male			Female		
	n	Mean	S.D.	*n*	Mean	S.D.
Age						
Unexposed	14	31.6	4.8	24	30.8	6.1
Exposed	15	28.5	6.0	26	27.2	6.0
Tobacco use						
Unexposed	11 (78.6%)			0		
Exposed	13 (86.7%)			0		
Among smokers						
Pack years unexposed	11	7.1	5.2	0		
Pack years exposed	13	6.1	8.4	0		
Among exposed						
Duration (years) butadiene exposure	15	8.6	6.1	26	6.5	5.5

the women in either group smoked, while tobacco use was common among exposed and unexposed men. The mean pack-years of tobacco use among smokers was 6.1 in butadiene-exposed workers and 7.1 among unexposed comparison subjects. Among butadiene-exposed workers, men and women were exposed to BD for a similar number of years.

Personal air samples were available for 40 exposed subjects, among whom 20 had measurements taken on two separate days. Using all available measurements, for polymer and DMF analysts the median air levels were 1.0 and 3.5 ppm, respectively. Among recovery operators, air levels of 1.1 ppm were found during routine activities, while the median air level during pump repair and related operations was 45 ppm.

It was possible to establish cultures and assess M_f values for 61 (77.2%) subjects (unexposed % 29, exposed = 32). Cloning efficiency was similar for butadiene-exposed (29%) and unexposed subjects (33%) (P % 0.51), but was negatively correlated with M_f ($r = -0.25$, $P = 0.06$) and significantly lower among women than men ($P \leqslant 0.01$).

The mean (X) and median M_f were similar in unexposed and butadiene-exposed subjects ($P = 0.68$), with similar results for men ($P = 0.75$) and women ($P = 0.56$) (Table 3). Maximum M_f could be estimated for an additional seven subjects, of whom five (unexposed % 3, exposed = 2) scored less than the median M_f (median $M_f = 16.9 \times 10^{-6}$). Including these subjects, no differences were found in the proportion of unexposed and exposed subjects above the median M_f ($\chi^2 = 0.0009$, $P = 0.98$).

M_f were non-significantly increased ($P = 0.12$) in women (median $M_f = 17.6 \times 10^{-6}$) compared to men (median $M_f = 14.5 \times 10^{-6}$), with similar differences among unexposed ($P = 0.29$) and butadiene-exposed subjects ($P = 0.25$). M_f increased significantly with age ($r = 0.29$, $P = 0.02$), with similar although non-significant associations among unexposed ($r = 0.29$, $P = 0.13$) and butadiene-exposed ($r = 0.25$, $P = 0.17$). Among male smokers, no associations were seen of M_f with amount smoked ($r = 0.05$, $P = 0.83$) or total pack-years of tobacco use ($r = 0.05$, $P = 0.82$). However, most tobacco users had low-level exposure to tobacco smoke (64% used $\leqslant 10$ cigarettes per day and 82% had $\leqslant 10$ pack-years of total consumption).

M_f was not significantly related, in the butadiene-exposed, to butadiene exposure ($r = 0.28$, $P = 0.16$), as determined on the day of biologic sample collection, nor to duration of employment in butadiene-associated jobs ($r = 0.06$, $P = 0.75$).

After adjustment by multiple regression analysis for age, sex, and cloning efficiency, the adjusted mean M_f (X_{adj}) was 13.6×10^{-6} in unexposed and 18.0×10^{-6} in butadiene-ex-

Table 3
Hypoxanthine-guanine phosphoribosyl transferase (*hprt*) mutation frequency (M_f) in butadiene-exposed and unexposed workers (Yanshan, China, 1994)

	Unexposed					Exposed					*P* value	
Subjects	*n*	X^a	95% CI	Median	X^b_{adj}	*n*	X^a	95% CI	Median	X_{adj}	M-W U[c]	Regression[d]
Total	29	20.2	14.5–26.0	16.9	13.6	32	21.6	15.0–28.2	16.9	18.0	0.68	0.13
Female	19	22.7	14.5–30.9	17.4	16.4	18	25.1	13.9–36.2	18.9	24.0	0.56	0.11
Male	10	15.5	8.7–22.2	13.9	11.8	14	17.2	11.2–23.1	14.5	13.9	0.75	0.51

[a] X, mean $M_f \times 10^{-6}$.
[b] X_{adj}, mean $M_f \times 10^{-6}$, adjusted by linear regression of $\ln(M_f)$ on age, sex, cloning efficiency, and exposure status.
[c] M-W U, P value of the Mann-Whitney U-test.
[d] Regression, P value for exposure status in linear regression on $\ln(M_f)$.

posed (Table 3). This regression-adjusted 32% difference was, however, not statistically significant ($P = 0.13$). Among women, the adjusted mean M_f (X_{adj}) was 16.4×10^{-6} among unexposed and 24.0×10^{-6} among butadiene-exposed, a statistically non-significant ($P = 0.11$) difference of 46%. Among men, the adjusted mean M_f (X_{adj}) was 11.8×10^{-6} among unexposed and 13.9×10^{-6} among butadine-exposed ($P = 0.57$). Adjustment, in men, for tobacco use did not alter the result.

Discussion

Overall, M_f was similar in unexposed (mean $M_f = 20.2 \times 10^{-6}$) and butadiene-exposed (mean $M_f = 21.6 \times 10^{-6}$) workers; M_f was not related to duration employed in exposed jobs. M_f decreased with cloning efficiency, increased with age, and was moderately greater in women than in men. After statistical adjustment for these factors, a non-significant ($P = 0.13$) increase in M_f of 32% was observed, due largely to greater M_f among exposed women ($P = 0.11$).

Ward et al. (1994), using an autoradiographic procedure, found a greater than 3-fold increased mutation frequency in butadiene-exposed ($M_f = 3.84 \times 10^{-6}$, $n = 8$) vs. unexposed ($M_f = 1.03 \times 10^{-6}$, $n = 6$) workers in the U.S. The results of the U.S. study are updated in this volume (Ward, 1996). Several possible reasons could account for the difference in M_f findings between the U.S. and Chinese studies. Both studies are small, such that statistical sampling variability could account for at least some of the difference. Exposure estimates appear similar for both the U.S. and China study groups, but long-term exposure fluctuations were not characterized in either study. Our observation that process operators had high exposures sporadically indicates the need for long-term exposure monitoring, in this setting. The autoradiographic method showed increased mutation frequency among smokers (Ward et al., 1994), whereas we did not see such an effect. Findings for smoking effects on M_f, as measured by the *hprt* clonal assay, have not been consistent (Robinson et al., 1994), but the lack of association found here could have been due to the low level of tobacco use among smokers in our study. Further, the M_f values for this study population are generally elevated compared to our historical database (Robinson et al., 1994), indicating that other factors, possibly including life style and metabollic differences, may be operating.

It is possible that the cloning assay is less sensitive to environmental carcinogens, or, due to differences in cell culture methodology (Albertini et al., 1990; Ward et al., 1994), the cloning and autoradiography methods for M_f determination may be measuring mutation frequency in different sub-populations of lymphocytes. The autoradiography assay provides a measure of *hprt* mutation frequency only in those cells that proceed into or through *S*-phase during the 40-h in vitro culture. The cloning assay measures the M_f in a larger fraction of cells, including all cells that can form colonies, even if they entered *S*-phase after 40 h. Chronic exposure to a cytotoxic chemical, such as BD, may modify the 'normal" lymphocyte distribution or mutate a sub-population of T-lymphocytes. These mutant cells may be preferentially detected in the short-term 40-h assay, if they enter *S*-phase more rapidly. Further studies are needed to characterize the lymphocyte population assessed in these alternative methods. Clearly, a future study of an exposed population should employ both assays to address the different results.

Chronic exposure to a genotoxic agent may result in moderate increases in M_f, which fall within the range of the known heterogeneity of human M_f values (Robinson et al., 1994). This limitation might be clarified when studies to determine individual exposure, such as BD macromolecular adduct formation, are complete. Increases in M_f resulting from exposure to BD may be accompanied by specific mutation events, as reported in studies of mice (Cochrane and Skopek, 1994). This can possibly be evaluated by analysis of mutant colonies in this study.

Butadiene exposure was associated with a modest, if any, increase in *hprt* M_f in this population of Chinese workers. Other investigations are continuing to characterize biologic exposure

to BD in this population and to relate this exposure to specific mutations in *hprt* and to other cytogenetic events.

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